Enhanced Myosin Function Due to a Point Mutation Causing a Familial Hypertrophic Cardiomyopathy

Richard L. Moss, Jose Sant’Ana Periera

Most cases of familial hypertrophic cardiomyopathy have been attributed to mutations in thick or thin filament proteins in the myofibrils of myocardial cells. In a minority of cases, particularly those involving myosin binding protein C, such mutations have been associated with enhanced myofibrillar function, which has been inferred from changes in force and the kinetics of force development and relaxation in isolated muscle preparations and from changes in the dynamics of pressure development in working hearts. In most cases, such as those involving thin filament regulatory proteins or subunits of myosin, familial hypertrophic cardiomyopathy (FHC) mutations have been associated with depressed function in working hearts in living or skinned myocardium or in biochemical and other in vitro assays of actomyosin interaction. Whether myofibrillar function is enhanced or depressed, the hearts of affected individuals undergo hypertrophy as an adaptive response to altered myocardial function, and for particularly malignant mutations, there is a significantly increased probability of premature death because of mechanical or electrical abnormalities of the heart.

Although several FHC mutations have been identified using gene mapping approaches, little is known about the effects of most of the mutations on myofibrillar function or integrated contractile function in the context of human intact cardiac myocytes, muscle strips, or working hearts. However, in the case of one of these mutations, R403Q in the human β-myosin heavy chain (MHC), recent studies have taken advantage of the fact that mammalian slow muscle MHC is identical to cardiac β-MHC to obtain muscle that was heterozygous for the R403Q mutation. Biopsies from soleus muscles of individuals yielded fibers on which mechanical measurements could be made. Fibers from R403Q heterozygotes exhibited less force and slower shortening velocities than fibers from healthy homozygous individuals. The R403Q mutation has also been found to slow sliding velocities in in vitro motility assays of myosin from human soleus muscles and in other myosins with the R403Q substitution. In the context of these results, concentric hypertrophy is a plausible compensatory mechanism for reestablishing the work capacity and power output of heterozygous R403Q hearts toward normal.

R403Q Mutation Increases Force and Speeds Actin-Activated Cycling Kinetics of Myosin

In the present issue of Circulation Research, Tyska et al report results of an elegant study designed to assess the molecular mechanism of altered function caused by the R403Q mutation. Their work involves characterization of the ATPase activity and in vitro motility of mouse α-MHC expressed with the R403Q mutation, as well as measurements of the force-generating properties of single myosin molecules from homozygous mice expressing the mutation. This intriguing mouse model was previously developed by Seidman and colleagues, who also characterized cardiac function in heterozygous animals, and found that rates of pressure development were accelerated and rates of relaxation were slowed compared with healthy homozygous controls. In contrast to these earlier studies, Tyska et al found that ensembles of R403Q myosin yielded in vitro sliding velocities, actin-activated ATPase activities, and average forces that were greater than control values, whereas the ATPase activity of R403Q myosin alone (in the absence of actin) was not different from control. These results suggest that the R403Q mutation accelerates the kinetics of myosin interaction with actin, an idea that is further supported by the authors’ findings that unitary force and mean step size in single molecules did not differ between R403Q and control myosins. However, when the authors measured the average duration of force generating events (t_m), no differences were observed between R403Q and control myosins. As the authors point out, the solution to this conundrum might be found in the significantly different concentrations of MgATP used to assess in vitro motility and function of single myosin molecules. It is likely that the rate of MgADP dissociation determines crossbridge detachment rate in the in vitro motility assay where [MgATP] is high (mmol/L), whereas the rate of MgATP association determines crossbridge detachment rate in the optical trap assay (μmol/L MgATP). Thus, the rate of MgADP release might be faster for R403Q than for wild-type myosin, which is suggested by its greater ATPase activity, but the temporal resolution of the optical trap assay is insufficient to allow the increase in [MgATP] that would be required to detect the difference. Such a technical limitation in assessing t_m could certainly explain the apparent similarity in turnover kinetics between single molecules of R403Q and wild-type myosins but also suggests that if firm conclusions are to be drawn, alternative methods for assessing the rates of MgADP release in solution should be used in studies of the mutation.

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From the Department of Physiology, University of Wisconsin Medical School, Madison, Wis.

Correspondence to Richard L. Moss, PhD, 1300 University Ave, Madison, WI 53706. E-mail rlmos@physiology.wisc.edu


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Implications of Results for Mechanism of Myocardial Hypertrophy

Regardless of the precise molecular mechanism underlying the present observations, Tyska et al. have clearly shown a gain of function caused by the R403Q mutation. This is a potentially very important result, as the authors point out, that suggests that the hypertrophic response in individuals expressing this mutation is not due to depressed force or kinetics of contraction but instead is related to chronically increased energetic demands on the myocardium. In such a model, compensatory hypertrophy would increase tissue mass and thereby reduce wall stress and energy utilization per unit volume of myocardium. The observation that homozygous R403Q mice do not live for more than a few days after the transition from β-MHC to R403Q α-MHC (by ~4 days of age) suggests that the transition is too rapid or too great to be offset by the compensating effects of myocardial hypertrophy.

Although faster crossbridge cycling in humans and animals expressing the R403Q mutation could account for the faster rate of rise in pressure (+dP/dt) that has been observed in vivo and the faster rate of force development in muscle strips, faster kinetics do not straightforwardly explain the slower rate of relaxation of pressure (or myocardial force) observed in these same individuals. However, as discussed by the authors, the greater average force observed in R403Q myosin is most likely the result of a greater fraction of the duty cycle of myosin being spent in the force-generating state. This explanation seems plausible because $t_{on}$ is similar in R403Q and wild-type myosins, but total cycle time is shorter in R403Q myosin. Thus, the fraction of crossbridges bound to the thin filament at any given time should be greater for R403Q myosin, which in turn would be expected to enhance crossbridge-induced cooperative activation of the thin filament and slow the rate of relaxation. A cooperative mechanism similar to this has recently been proposed as an explanation for the slower rates of relaxation observed in mouse hearts expressing significant amounts of β-tropomyosin on a normal α-tropomyosin background.

Discrepancies With Earlier Studies

The gain of function results presented by Tyska et al. are consistent with the hemodynamic phenotypes of both humans and animals expressing the R403Q mutation, as discussed in the preceding paragraph. However, the results do not seem to agree with the findings by Lankford et al., in which the contractile performance of skinned soleus fibers from patients heterozygous for the R403Q mutation was found to be depressed. Neither do they agree with findings by Cuda et al., in which the in vitro sliding velocity of human R403Q myosin was slower than normal. Assuming that all measurements are free from artifact and that the assay systems use similar concentrations of myosin, there are several issues that might account for the discrepancies and would need to be resolved by additional measurements. Foremost among these is the need to standardize measurement conditions to ensure that thin filaments have similar compositions. In the measurements of in vitro sliding velocities by Tyska et al. and Cuda et al., the thin filaments are unregulated, whereas in the measurements of mechanical properties of human soleus muscles, the thin filaments are regulated but do not contain cardiac isoforms of troponin I and troponin T. Such considerations are important because it is clear that the presence of regulatory proteins can affect mechanical properties in the in vitro motility assay and that different isoforms of regulatory proteins have varying effects on the kinetics of crossbridge interaction with actin. A second possibility, suggested by Tyska et al., is that the ability to detect enhanced function in the R403Q mutant is related to the use of native myosin as opposed to heavy meromyosin. However, this would not explain the depressed function seen by Cuda et al., who also used whole myosin in their motility assay, or by Lankford et al. in skinned fibers expressing R403Q myosin. Another possibility is that the results obtained by Tyska et al. are related to the expression of the R403Q mutation in mouse α-MHC, which has faster kinetics than β-MHC normally expressed in humans. Although it might be possible to address this point directly by expressing the R403Q mutation on a mouse β-MHC background, questions could still be raised owing to the 3- to 4-fold faster kinetics of mouse β-MHC compared with human β-MHC. Tyska et al. are certainly aware of this issue and make the important point that they have observed similar gain of function in cardiac myosin obtained by biopsy from FHC patients, although this work has yet to be published, and the nature of the underlying mutation is unclear.

Looking to the Future

Overall, Tyska et al. have shown that there is acceleration of actin-activated cycling kinetics of mouse α-MHC expressing the R403Q mutation. Such a gain of function is likely to stimulate the development of compensatory hypertrophy via pathways that somehow differ from the pathways involved in hypertrophy as a response to a loss of function, ie, reduced power-generating capabilities. At the present time, the mechanism for the enhancement of myosin function is not known for certain, although it seems likely that the rate of actin-activated nucleotide turnover by myosin is accelerated by the R403Q mutation. New experimental approaches will be required to resolve this point, which would be most directly done by assessing MgADP dissociation rates from R403Q and wild-type myosin. Furthermore, this study has used a unique and important mouse model of a human familial hypertrophic cardiomyopathy to study mechanisms of altered function; ie, homozygous expression of this mutation in mouse α-MHC has yielded pure preparations of mutant myosin for studies of force-generating and kinetic properties. Looking to the future, there is a growing need to study the R403Q mutation in the context of regulated thin filaments from the heart, both to investigate the effects of cardiac regulatory proteins on actin activation of myosin turnover kinetics and to determine whether variations in regulatory protein content of the thin filament might account for qualitative differences in results obtained by different investigators studying the R403Q mutation. Ultimately, experiments that assess force and sliding velocities of human β-MHC and regulated thin filaments from the heart will be useful in providing validation of mouse models of FHC and the
subsequent use of these models in studies of interventions designed to slow or reverse development of the hypertrophic phenotype.

References

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